Curdlan Quantification :

Overview :

Curdlan and other (1→3)-β-D-glucans specifically bind the triphenylmethane dye, aniline blue, and a benzophenone fluorochrome found in the dye mixture. Calcofluor and Congo Red also bind to curdlan and induce fluorescence, but these dyes are not specific for (1→3)-β-glucans. So we used aniline blue dye to quantify our curdlan production from *E.coli* and *S.cerevisiae*.

Procedure :

References : 1,3--Glucan Quantification by a Fluorescence Microassay and Analysis of Its Distribution in Foods ; YUAN-TIH KO AND YU-LING LIN, J. Agric. Food Chem. 2004, 52, 3313−3318*

1. Prepare DYE MIX :
   - 40 volumes of 0.1% aniline blue in water
   - 21 volumes of 1 N HCl
   - 59 volumes of 1 M glycine/NaOH buffer pH 9.5

2. Diluted sample (also Curdlan Standard) 10-50-fold with1 N NaOH to a final volume of 300 µL in a 1.5 mL microcentrifuge tube

3. Add 30 µL of 6 N NaOH

4. Incubated at 80 °C for 30 min

5. The tube was immediately put on an ice bath

6. Add 630 µL DYE MIX into the tube and mix

7. Incubated at 50 °C for 30 min

8. The unbound fluorescent dye was decolorized at room temperature for 30 min

9. Mesure the fluorescence intensity with a fluorescence spectrophotometer
   - emission wavelength of 502 nm (20 nm slit width)
   - excitation wavelength of 398 nm (20 nm slit width).